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Evaluation of some hybrid combinations for exploitation of two line system heterotic in Rice (*Oryza sativa* L.)

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ABSTRACT:
Photo-thermosensetive genic male sterile (PTGMS) lines of rice have tremendous potential in realizing further quantum yield and economical hybrid rice seed production cost. The study was conducted during two rice growing seasons of 2015 and 2016 at the Experimental Farm of the Rice Research and Training Center (RRTC), Sakha, Kafr El-Sheikh, Egypt. Ten hybrid rice combinations derived from hybridization of one elite and adapted Japonica PTGMS line (PTGMS-38) and ten Indica, Japonica and Indica Japonica genotypes along with two check (Giza 178 and Giza 179) were used to analysis of genetic parameters and standard heterosis for 10 traits. Highly significant positive standard heterosis over the best local check varieties Giza 178 and Giza 179 for grain yield/plant was observed in all hybrids. The hybrids, PTGMS-38 x Sakha 106 (H10), PTGMS-38 x GZ8479 (H9), PTGMS-38 x Chinese 2 (H8), PTGMS-38 x Giza 177 (H6) and PTGMS-38 x Sakha 101 (H7) with standard heterosis of 54.47%, 54.03%, 51.63%, 50.98% and 49.24%, respectively over the best local check variety Giza 179 were considered as promising. Hence, these hybrid rice combinations may be used for commercial exploitation of two line hybrid rice breeding. Mean sum of squares due to genotypes showed significant differences for ten traits studied, indicating the presence of high genetic variability among the genotypes. The estimates of GCV were lower than the respective PCV, indicating the influence of environmental factor on the expression of the studied traits. Characters like, spikelet fertility % (99.12%, 47.21%), grain yield/plant (98.30%, 36.11%), plant height (99.20%, 35.28%), panicle weight (97.30%, 35.27%) and flag leaf area (89.32%, 31.97%) showed high heritability coupled with moderate genetic advance as percent of mean, suggesting that selection for the improvement of these characters may be rewarding and also greater role of non-additive gene action and suggesting heterosis breeding could be used to improve these characters. Based on ten studied characters, the genotypes were grouped into three clusters at 0.98% similarities coefficient.
Key words: *Oryza sativa* L., PGMS, genetic variability, heterosis

INTRODUCTION

Hybrid rice technology is one such innovative breakthrough that can further increase rice production leading to food security and reduction of poverty in Egypt. This technology can be used to break the current yield plateau in rice, where yield levels of the conventional cultivars released have stabilized [1].

Commercial hybrids typically yield 15-20% more than the best inbred varieties grown under similar conditions believed to be the result of "hybrid vigor" or "heterosis" from crossing the two parents [2]. The heterosis advantage of hybrids may be expressed by superiority over inbred varieties in grain yield, vigor, panicle size, number of spikelets per panicle, and number of productive tillers.

Hybrid varieties are generally developed by the "three-line" or the "two-line" breeding method. The cytoplasmic genetic male sterility system is a three-line system involving a CMS source, a maintainer and a restorer is extensively being used in the production of commercial rice hybrids [3, 4]. In the two-line system, certain lines, referred to as S lines, can be either male sterile (functionally female) or male (produces viable pollen) depending upon temperature and day length.

Under one set of temperature/ day length combination, the S lines are crossed as females to fertile inbred lines to produce hybrid seed, while under separate temperature/day length combination, the same lines are allowed to self-pollinate and produce viable seed to maintain a source of the line.

The objectives of this investigation are to evaluate ten promising hybrid combinations derived from two-line system for some agronomic and yield and its component traits for may be used for commercial exploitation of two line hybrid rice breeding.

MATERIALS AND METHODS

The experiment was conducted at the experimental farm of Rice research and training center (RRTC), Sakha, Kafr El-Sheikh, Egypt during the two successive rice seasons 2015 and 2016. The experimental materials used for study consisted of

one newly developed and well adapted photo-thermo sensitive genic male sterile line (PTGMS-38) as female and 10 testers; Giza 178, GZ6296, PR 78, Giza 179, Large stigma, Giza 177, Sakha 101, Chinese 2, GZ8479 and Sakha 106 were used as male parents to get 10 F1 hybrids in line x tester combinations Table 1.

Hybrids were evaluated along with parents and two check commercial rice varieties (Giza 178 and Giza 179) in a Randomized Complete Block Design with three replications. Thirty-day old seedlings were transplanted in 5 m. long; seedlings were into 20 x 20 cm spacing and one seedling/hill. Each test genotype consisted of three rows of 5m length. Observations were recorded on five plants plant-1 taken at random from each entry in each replication for ten agronomic and yield and its component traits;

Days to heading (day): It was determined as number of days from sowing to the time when more than 50% of panicles emerged.

Plant height (cm): Measured on the main clum from ground level to the tip of the panicle in centimeter (cm).

Number of tillers/plant: Total numbers of tillers were counted on each plant at the time of maturity

Number of panicles/plant: Counted as number of panicles bearing tillers per plant at the maturity stage.

Flag leaf area (cm^2): Estimated at the pre-flowering stage following the formula reported by [5] as follows:

Flag leaf area (cm^2) = K X length (cm) X width (cm).

Where: K (0.75) is a correction factor.

Panicle length (cm): The main panicle was measured from panicle base up to apiculus of the upper most spikelet of the panicle.

Panicle weight (g): Defined as weight of the main panicle for each plant.

1000-grain weight (g): It was measured as weight of 1000 random filled grains per plant.

Spikelet fertility%: Ratio of the number of fertile spikelets to the total number of spikelets per panicle.

Grain yield/plant (g): Weight of grain yield of each individual plant, then adjusted to 14 % moister content.

Statistical analysis: Analysis of variance was computed by IRRISTAT program. Correlation coefficients (r) among all studied traits were computed using SPSS statistical package according to [6]. Estimates of genotypic variance (σ^2g), environmental variance (σ^2e), genotypic x environmental variance (σ^2gy), phenotypic variance (σ^2ph), genotypic (GCV), phenotypic (PCV) coefficient of variation components and the expected genetic advance from selection (Δg %) according to the formula suggested by [7] as follows:
Genotypic variance (σ^2g) = M1-M2/ry
Environmental variance (σ^2e) = M3
Phenotypic variance (σ^2ph) = σ^2g + σ^2gy+ σ^2e
Where: M1= Mean square due to varieties within treatment,
M2 = Mean square due to varieties x year interaction,
M3 = Mean squares due to error, and r = number of replications

$$\textbf{Phenotypic coefficient of variability (P.C.V.)} = \frac{\sqrt{Ph}}{X} \times 100$$

$$\textbf{Genotypic coefficient of variability (GCV)} = \frac{\sqrt{g}}{X} \times 100$$

Other genetic parameters i.e., heritability (H^2) and genetic advance upon selection (ΔG) were calculated as follows:
Heritability (H^2): was estimated as the percent ratio of genotypic variance to phenotypic variance [8].

$$H2b\ \% = \frac{\sigma 2g}{\sigma 2Ph} \times 100$$

Genetic advance upon selection (ΔGs) as percent of the mean (ΔG %) were computed according to [9] as follows:

$$\Delta Gs = K*H^2_b*\sigma Ph$$

$$\Delta G\% = \frac{\Delta G}{X} \times 100$$

Where K is the selection differential and equals 2.06 at selection intensity of 5%.

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Table 1. Photo-thermosensitive genic male sterility lines (PTGMS) and tester lines/varieties used for the experiment.

Photo-thermosensitive genic male sterile line		
Line	**Source**	**Origin**
PTGMS – 38	Cross breeding Nongken 58s/Sakha101 then acquired by anther culture	Egypt
Tester Lines		
Testers	Parentage	Origin
Giza 178	Giza175/Milyang 49	Egypt
GZ6296	AC1225/Hualien Yu 202	Egypt
PR 78	IR58025A/Pusa basmati-1	China
Giza 179	GZ1368-5-S-5/GZ6296-12-1-2-1-1	Egypt
Large stigma	Unknown	China
Giza 177	Giza 171/Yomjo No. 1//PiNo.4	Egypt
Sakha 101	Giza 176/Milyang 79	Egypt
Chinese 2	Unknown	China
GZ8479	Gz6214/EMPSSK 104	Egypt
Sakha 106	Giza 177/Hexi 30	Egypt

Table 2. Mean values of hybrid combinations for various agro-botanical traits and standard heterosis over two commercial rice varieties.

No.	Hybrid combinations	Measured Traits										Yield advantage over Standard checks (%)	
		DH	PH	NTP	NPP	FLA	PL	PW	1000 GW	SF %	GYP	Giza178	Giza179
1	PTGMS-38/Giza 178 (H1)	122.3	129.7	23.0	21.7	44.1	21.4	6.1	28.4	65.6	54.6	22.70**	18.95**
2	PTGMS-38/Gz6296 (H2)	111.0	135.0	19.3	18.7	48.4	23.3	6.0	29.6	73.4	62.4	40.22**	35.95**
3	PTGMS-38/PR78 (H3)	127.0	157.3	28.0	26.7	48.8	21.9	5.5	29.4	68.8	55.5	24.72**	20.92**
4	PTGMS-38/Giza 179 (H4)	113.0	139.3	21.3	20.0	49.0	22.0	4.6	29.5	45.6	48.7	9.44**	6.10**
5	PTGMS-38/Large Stigma (H5)	125.3	138.7	20.0	19.0	47.2	24.9	5.5	35.2	47.2	49.5	11.24**	7.84**
6	PTGMS-38/Giza 177 (H6)	105.7	105.0	22.2	21.0	29.3	20.0	5.6	29.3	96.4	69.3	55.73**	50.98**
7	PTGMS-38/Sakha 101 (H7)	120.0	105.0	25.0	23.3	39.5	20.4	6.3	29.6	82.4	68.5	53.93**	49.24**
8	PTGMS-38/Chinese 2 (H8)	112.7	114.0	20.0	19.3	36.2	19.1	7.3	27.8	88.3	69.6	56.40**	51.63**
9	PTGMS-38/GZ8479 (H9)	109.0	94.3	20.8	19.3	35.3	18.6	6.4	27.6	94.5	70.7	58.88**	54.03**
10	PTGMS-38/Sakha 106 (H10)	101.0	102.0	22.1	20.5	36.3	19.7	7.4	28.8	94.5	70.9	59.33**	54.47**
11	Giza 178 (Check)	106.0	105.0	23.9	22.8	38.1	22.3	4.3	21.5	89.5	44.5	--	--
12	Giza 179 (Check)	93.0	98.0	24.8	23.4	33.5	21.7	4.6	27.5	90.8	45.9	--	--
L.S.D. 0.05 % 0.01 %		1.29 1.88	2.58 3.77	2.21 3.23	2.03 2.97	3.22 4.71	0.74 1.09	0.24 0.34	0.36 0.52	2.37 3.46	1.93 2.82	--	--

Abbreviations: DH, Days to heading (day); PH, Plant height (cm); NTP, Number of tillers/plant; NPP, Number of panicles/plant; FLA, Flag leaf area (cm^2); PL, Panicle length (cm); PW, Panicle weight (g); 1000-GW, 1000-grain weight (g); SF %, Spikelets fertility % and GYP, Grain yield/plant (g).

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Table nr. 3. Mean squares of twelve rice genotypes for agro-morphological and yield traits under study.

S.O.V.	d.f.	Sum of mean square									
		DH	PH	NTP	NPP	FLA	PL	PW	1000 GW	SF %	GYP
Replications	2	0.08	1.44	1.03	4.26	0.68	0.29	0.07	0.05	0.18	0.11
Genotypes	11	311.67**	1251.57**	19.41**	16.97**	137.87**	9.94**	3.07**	27.46**	972.37**	330.22**
Error	22	0.84	3.38	2.48	2.10	5.29	0.28	0.03	0.07	2.86	1.89

Abbreviations: DH, Days to heading (day); PH, Plant height (cm); NTP, Number of tillers/plant; NPP, Number of panicles/plant; FLA, Flag leaf area (cm^2); PL, Panicle length (cm); PW, Panicle weight (g); 1000-GW, 1000-grain weight (g); SF %, Spikelets fertility % and GYP, Grain yield/plant (g).
**significant at 1% probability level,

Table nr. 4. Grand mean, range, Variance components, estimates of phenotypic (PCV) and genotypic (GCV) coefficient of variation, heritability (h^2 %) and genetic advance for 10 characters in rice

Studied traits	Grand mean	Range	Variance components			Coefficient of variation		Heritability (h^2 %)	Genetic Advance	
			$\sigma^2 g$	$\sigma^2 p$	$\sigma^2 e$	GCV	PCV		G.S.	G.S. (%)
Days to heading (day)	112.2	93.0-127.0	103.61	104.45	0.84	9.07	9.11	99.19	20.88	18.61
Plant height (cm)	118.6	94.3-157.3	416.06	419.44	3.38	17.20	17.27	99.20	41.84	35.28
Number of tillers/plant	22.5	19.3-28.0	5.64	8.13	2.48	10.54	12.65	69.46	4.07	18.09
Number of panicles/plant	21.3	18.7-26.7	4.95	7.06	2.10	10.44	12.47	70.19	3.84	18.02
Flag leaf area (cm2)	40.5	29.3-49.0	44.19	49.48	5.29	16.43	17.38	89.32	12.94	31.97
Panicle length (cm)	21.3	18.6-24.9	3.22	3.50	0.28	8.44	8.80	91.98	3.54	16.67
Panicle weight (g)	5.8	4.30-7.40	1.01	1.04	0.03	17.36	17.60	97.30	2.04	35.27
1000-grain weight (g)	28.7	21.5-35.2	9.13	9.20	0.07	10.54	10.58	99.29	6.20	21.63
Spikelets fertility %	78.1	45.6-96.4	323.17	326.03	2.86	23.02	23.12	99.12	36.86	47.21
Grain yield/plant (g)	59.5	44.5-70.9	109.44	111.34	1.89	17.68	17.83	98.30	21.36	36.11

Table nr. 5. Estimates of simple correlation coefficients among pairs of the studied characters.

Traits	DH	PH	NTP	NPP	FLA	PL	PW	1000-GW	SF %	GYP
DH	1.00									
PH	0.729**	1.00								
NTP	0.118	0.094	1.00							
NPP	0.143	0.153	0.993**	1.00						
FLA	0.684**	0.892**	-0.011	0.026	1.00					
PL	0.335	0.624*	-0.033	0.018	0.677**	1.00				
PW	0.106	-0.191	-0.319	-0.343	-0.196	-0.570*	1.00			
1000-GW	0.486	0.466	-0.272	-0.283	0.396	0.358	0.232	1.00		
SF %	-0.654**	-0.818**	0.127	0.107	-0.868**	-0.708**	0.324	-0.570*	1.00	
GYP	-0.042	-0.367	-0.263	-0.297	-0.414	-0.730**	0.851**	0.130	0.526*	1.00

Abbreviations: DH, Days to heading (day); PH, Plant height (cm); NTP, Number of tillers/plant; NPP, Number of panicles/plant; FLA, Flag leaf area (cm^2); PL, Panicle length (cm); PW, Panicle weight (g); 1000-GW, 1000-grain weight (g); SF %, Spikelets fertility % and GYP, Grain yield/plant (g).

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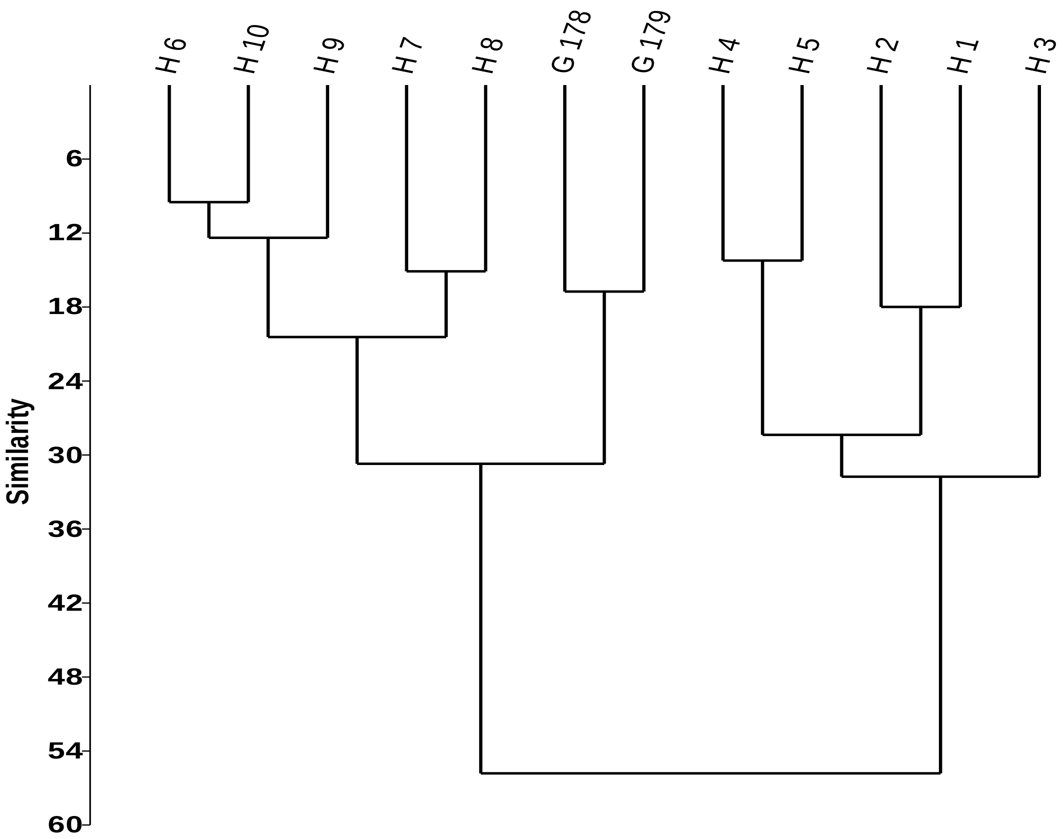

Figure 1. Dendogram of ten hybrid combinations and two commercial rice varieties based on ten quantitative traits

RESULTS AND DISCUSSION

Mean performance: The data pertaining to the mean performance of F1 rice hybrids for various quantitative characters is presented in Table 2. Early maturing hybrids are desirable as they produce more yield/day and fit well in multiple cropping systems [10]. For days to heading ranged from 93.0 days to 127.0 days with the mean of 112.2 days. The check variety Giza 179 (93.0 days) and hybrid PTGMS-38/Sakha 106 (101.0 days) were noted to be early maturing varieties, while PTGMS-38/PR78 (127.0 days) was a late maturing hybrid. Selection of rice genotypes with appropriate plant height and non-lodging characteristic is important for high yield potential hybrids [11] have suggested use of dominant. Highest plant height was observed in hybrids PTGMS-38/PR78 (157.3 cm) and PTGMS-38/Giza 179 (139.3 cm) and the lowest value observed for hybrid PTGMS-38/GZ8479 (94.3 cm). For number of tillers/plant mean performance ranged from 19.3 to 28.0 tillers with the mean of 22.5 tillers. The hybrid combination PTGMS-38/PR78 recorded the highest mean value (28.0 tillers). Hybrids are generally characterized by having longer panicles indicating their efficiency in partitioning of assimilates to reproductive parts [10]. Among the hybrids investigated, the number of panicles/plant ranged from 18.7 to 26.7. The hybrid PTGMS-38/PR78 was exhibited highest panicles/plant and least was found in PTGMS-38/Gz6296 while check Giza 178 and Giza 179 exhibited 22.8 and 23.4 panicles, respectively. Concerning flag leaf area trait, the hybrid combinations PTGMS-38/Giza 179 and PTGMS-38/PR78 gave the highest mean values for this trait with 49.0 and 48.8 cm^2, respectively.

Panicle length was exhibited highest in PTGMS-38/Large Stigma (24.9 cm) and lowest in PTGMS-38/GZ8479 (18.6 cm). The trait panicle weight was varied from 4.3 g (in Giza178) to 7.4 g (in PTGMS-38/Sakha 106). Hybrid PTGMS-38/Large Stigma was exhibited highest 1000-grain weight (35.2 g), while Giza 178 and Giza 179 both were exhibited lowest (21.5 g and 27.5 g), respectively. All rice

hybrids were performed better yield than high yielding checks Giza 178 and Giza 179. Higher yield of hybrids resulted from their increased spikelets fertility %, which enhanced the sink capacity. [12] Reported that the average yield of F1 hybrid rice was 17% higher than that of indica inbreds. Yield/plant was recorded highest in PTGMS-38/Sakha 106 (70.9 g) and lowest in check Giza 178 (44.5 g).

Standard heterosis (Yield advantage over checks %): The success of hybrid rice programme depends upon the magnitude of heterosis which also helps in the identification of potential cross combinations to be used in the conventional breeding programmes to create wide array of variability in the segregating generations. Good hybrids should manifest high heterosis for commercial exploitation [13]. Standard heterosis of ten hybrid combinations for grain yield studied trait on the basis of the check varieties (Giza 178 and Giza 179) is presented in Table 2. The average of observed heterosis of each hybrid for this trait was compared with the standard varieties. Highly significant positive heterosis was observed in all hybrids in this trait. The highest values heterosis were recorded for hybrid combinations PTGMS-38/GZ8479 and PTGMS-38/Sakha 106 over the check varieties Giza 178 and Giza 179. Hence, these hybrids may be used for commercial exploitation of two line hybrid rice breeding.

Analysis of variance: Analysis of variance (ANOVA) of ten morphological and yield and its component traits exhibited in the Table 3. The differences within replications are not significant. The calculated values for genotypes with respect to ten traits are significance. Analysis of variance reveals that varietal differences are signified and wide variability present among the genotypes with respect to all the traits. From these results it was also noticed, that least significant difference revealed highly diversified genotypes for their performance and selection can be performed for various traits and this high level of variation strongly increases the efficiency of selection in breeding program. These results are in agreement with what published previously by [14, 15].

Estimates of genetic variability: Variance components: The results in Table 4 showed that phenotypic variance was higher than the genotypic variances for all the studied traits thus indicated the influences of environmental factor on these traits. Similar findings were earlier reported by [16, 17].

Coefficient of variability: The high genotypic coefficient of variability (GCV) and phenotypic coefficient of variability (PCV) recorded for spikelets fertility % indicate the existence of wide spectrum of variability for these traits and offer greater opportunities for desired traits through phenotypic selection (Table 4). Moderate PCV and GCV values recorded for grain yield/plant, panicle weight, plant height and flag leaf area while, rest of the characters recorded low PCV and GCV. The estimates of PCV were higher than GCV for all the traits. However, [18] reported small difference between GCV and PCV for all the characters under study, which indicated less influence of environment over expression of the traits.

Heritability and genetic advance: The results of heritability indicated that high heritability values were recorded for all studied traits Table 4. The expected genetic advance values for ten traits of the genotypes evaluated is presented in this table. These values are also expressed as percentage of the genotypes mean for each character so that comparison could be made among various characters, which had different units of measurement. High heritability along with high genetic advance is an important factor. High heritability coupled with high genetic advance was recorded for spikelets fertility % (99.12% and 47.21%), grain yield/plant (98.30% and 36.11%), plant height (99.20% and 35.28%) and panicle weight (97.30% and 35.27%). Thus it is interpreted that the characters viz., spikelets fertility %, grain yield/plant, plant height and panicle weight were controlled by additive gene action, which could be improved through simple selection methods. The characters showing high heritability with low genetic advance indicated the presence of non-additive gene action. Hence selection could be postponed for these characters or these characters could be improved by intermating of superior genotypes of segregation population from recombination breeding [19].

Correlation coefficient: Out of 15 significant estimates among the total 45 correlations obtained between different character pairs, 8 correlation coefficients were positive in nature while only 7 estimates were negative (Table 5). This represents highly favorable situation for obtaining high response to selection in improving yield and yield components in rice genotypes evaluated. The measure of degree of symmetrical association

between two variables or characters revealed that grain yield/plant had highly significant and positive correlation with panicle weight besides having significant and positive association with spikelets fertility %. Therefore, these characters emerged as most important associates of grain yield in rice. The spikelet fertility % exhibited strong negative association with days to heading, plant height, flag leaf area and panicle length. The flag leaf area had strong positive association with panicle length, which augurs well for providing correlated response during selection for improving these characters. The above observations of strong positive associations between yield and yield components are in agreement with the available literature in rice [20, 21, 22].

Cluster analysis: Cluster analysis was applied in the distance criteria of 0.98 grouped genotypes into three clusters (Fig. 1). Cluster I comprised of five hybrid combinations viz., H6, H7, H8, H9 and H10. These hybrids were similar in most of traits and high in grain yield/plant. Cluster II is the least cluster comprising two check varieties (Giza 178 and Giza 179) were placed in it. While there are the rest five hybrid combinations H1, H2, H3, H4 and H5 in cluster III. There are several studies on rice diversity and clustering using agronomic traits [23].

CONCLUSIONS

Presence of substantial heterosis and economic hybrid seed production are two most desirable components for success of any commercial hybrid rice breeding programme. Photo-thermosensetive genic male sterile (PTGMS) lines of rice in this regard have tremendous potential in realizing further quantum yield and economical hybrid rice seed production cost. It can be concluded from the present research studies that the hybrids, PTGMS-38 x Sakha 106, PTGMS-38 x GZ8479, PTGMS-38 x Chinese 2, PTGMS-38 x Giza 177 and PTGMS-38 x Sakha 101 with high standard heterosis over the best local check variety Giza 179 were considered as promising. Hence, these hybrid rice combinations may be used for commercial exploitation of two line hybrid rice breeding.

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